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Evolution and development of scyphozoan jellyfish

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ABSTRACT

Scyphozoan jellyfish, or scyphomedusae, are conspicuous members of many ocean ecosystems, and have large impacts on human health and industry. Most scyphomedusae are the final stage in a complex life cycle that also includes two intermediate stages: the larval planula and benthic polyp. In species with all three life-cycle stages, the metamorphosis of a polyp into a juvenile scyphomedusa (ephyra) is termed strobilation, and polyps can produce one ephyra (termed monodisc strobilation) or many ephyrae (termed polydisc strobilation). In contrast to species with planula, polyp and medusa stages, a handful of scyphozoan species possess modified life cycles with reduced or absent stages. The evolutionary patterns associated with strobilation and life-cycle type have not been thoroughly investigated, and many studies of ephyra development and strobilation induction are not yet synthesized. Herein, I place the development of scyphomedusae in an evolutionary context. I first review the current evolutionary hypotheses for Scyphozoa. Next, I review what is known about scyphomedusa development across a broad diversity of species, including the first signs of strobilation, the formation of strobila segments, and the morphogenesis of ephyrae. I then review cases where the canonical scyphozoan life cycle has been modified, and take advantage of phylogenetic hypotheses to place these observations in an evolutionary context. I show that the evolution of monodisc strobilation occurred at least twice, and that the loss of intermediate life-cycle stages occurred several times independently; by contrast, the reduction of the medusa stage appears to have occurred within a single clade. I then briefly review the major natural cues of strobilation induction. Finally, I summarize what is currently known about the molecular mechanisms of strobilation induction and ephyra development. I conclude with suggestions for future directions in the field.

Key words: Scyphozoa, Cnidaria, life cycle, metamorphosis, development, strobilation, complex life cycles, life cycle evolution, Medusozoa, jellyfish.

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I. INTRODUCTION

Scyphozoans (Cnidaria) are abundant and important members of many ocean habitats, and their most obvious ecological impact is related to the final life-cycle stage: the jellyfish. This jellyfish stage, termed a 'scyphomedusa' or 'medusa', is the most recognizable scyphozoan form, but it is far from the only one. Scyphozoan life cycles are among the most complex of any non-parasitic animal, with different life-cycle stages inhabiting different ecosystems and varying in size by orders of magnitude. These complex life cycles provide a unique opportunity to study life-cycle evolution, the role of development in the evolution of novel life histories, and the dynamics between development and the environment.

In most scyphozoans, the life cycle is broken into three stages. Scyphomedusae produce either eggs or sperm (rarely both; Morandini & Marques, 2010), which fuse and develop into ciliated larvae, termed 'planulae'. In most species, a planula settles to the benthos and metamorphoses into the sessile life-cycle stage, known as a 'scyphistoma', 'scyphopolyp', or most commonly just 'polyp'. Polyps resemble tiny sea anemones, and although polyps can be incredibly abundant, their ecological importance is only now becoming apparent (Lucas, Graham & Widmer, 2012). This is particularly true because, for most scyphozoans, it is the polyp stage that gives rise to the juvenile medusa, termed an 'ephyra', through a metamorphic process termed 'strobilation'. While polyps may go largely unnoticed, scyphomedusae have gained considerable attention in recent years due to their impact on people and ecosystems (Mills, 2001; Kawahara *et al.*, 2006; Lynam *et al.*, 2006; Purcell, Uye & Lo, 2007; Uye, 2008; Doyle *et al.*, 2013). However, much of the literature on medusa development and the mechanisms of strobilation is scattered and disconnected.

In this review, I synthesize information on medusa formation, life-cycle evolution, and the molecular mechanisms underlying medusa production in Scyphozoa.

(1) The potential impacts of scyphozoan life cycles

Scyphomedusae are recognizable animals that can have major impacts on ecology, economics, and human health. They play important roles as both predators and prey in many marine ecosystems (Doyle *et al.*, 2013). Scyphomedusae are preyed upon by a wide variety of animals, including the leatherback turtle *Dermochelys coriacea* (den Hartog & van Nierop, 1984) and the sunfish *Mola mola* (Pope *et al.*, 2010), as well as commercially important fish species, such as the Mediterranean *Boops boops* (Milisenda *et al.*, 2014). Scyphomedusae can have major impacts on human health and industry, particularly when they form mass aggregations or 'blooms'. Scyphomedusae can replace fish as top pelagic predators in overfished ecosystems (Lynam *et al.*, 2006), cause painful stings, and clog power-plant intakes (Mills, 2001; Purcell *et al.*, 2007). In a particularly extreme example, on December 10th, 1999, half of the Philippines lost power due to scyphomedusae caught in a power-plant cooling system (Mills, 2001).

Particular life-cycle features, such as strobilation type, are correlated with the potential to form blooms, and these life-cycle features are associated with different lineages of the scyphozoan phylogeny (Dawson & Hamner, 2009). Thus, understanding scyphozoan phylogenetic relationships, and what developmental features are associated with particular clades, is important to understanding many aspects of scyphozoan biology and ecology.

II. EVOLUTIONARY RELATIONSHIPS WITHIN *SCYPHOZOA*

The phylum Cnidaria contains two major clades: Anthozoa, which includes corals and sea anemones, and Medusozoa, which contains all medusa-forming species (Collins, 2002; although see Kayal *et al.*, 2013). Within Medusozoa, the monophyly of Scyphozoa has been recovered using morphological characters (Thiel, 1966; Marques & Collins, 2004), genes (Collins, 2002; Collins *et al.*, 2006), and transcriptomes (Zapata *et al.*, 2015), although there are exceptions (e.g. Werner, 1973; Kayal *et al.*, 2013). Medusozoa also contains the monophyletic groups Cubozoa (box jellies), Staurozoa (stalked jellies), and Hydrozoa (water jellies). The placement of these major non-scyphozoan groups is not well resolved (review in Collins *et al.*, 2006). Phylogenomic hypotheses suggest that Hydrozoa is the likely sister group to a clade containing Scyphozoa, Staurozoa and Cubozoa (Zapata *et al.*, 2015), but the relationships among the latter three groups are still unclear.

Within Scyphozoa, traditional Linnaean designations have been both challenged and supported in recent years using molecular phylogenetic methods. Traditionally, there are two major groups of scyphozoans: Coronatae and Discomedusae (Fig. 1); these two groups are well supported by current molecular phylogenies based on 18S and 28S rDNA (Collins *et al.*, 2006) and transcriptomic studies (Zapata *et al.*, 2015). Kayal *et al.* (2013) did not recover this topology using mitochondrial genomes, although the authors note that their results were not statistically well supported.

Coronate polyps live in firm, chitinous tubes (Jarms, Morandini & Da Silveira, 2002) (Fig. 2A), and coronate medusae have deep grooves along the bell margin, which separate thick ridges or 'pedalia' on the bell (Mayer, 1910; Marques & Collins, 2004). Each pedalium may or may not bear a single tentacle (Hyman, 1940). Coronate medusae can also be distinguished from other medusae by their non-pigmented oocytes (Marques & Collins, 2004; Daly *et al.*, 2007), simple mouth supported by a stalk (known as a 'manubrium'), and in many species by forward-facing tentacles.

In Discomedusae, polyps either lack a chitinous tube or possess a partial chitinous covering on the aboral stalk, into which the polyp cannot retract (Fig. 2B, C). Some polyps form cystic resting stages called 'podocysts' (Marques & Collins, 2004; Arai, 2009). Discomedusoid medusae have elaborate oral arms, a gastric system with canals (Marques & Collins, 2004), bells without grooves and pedalia (although

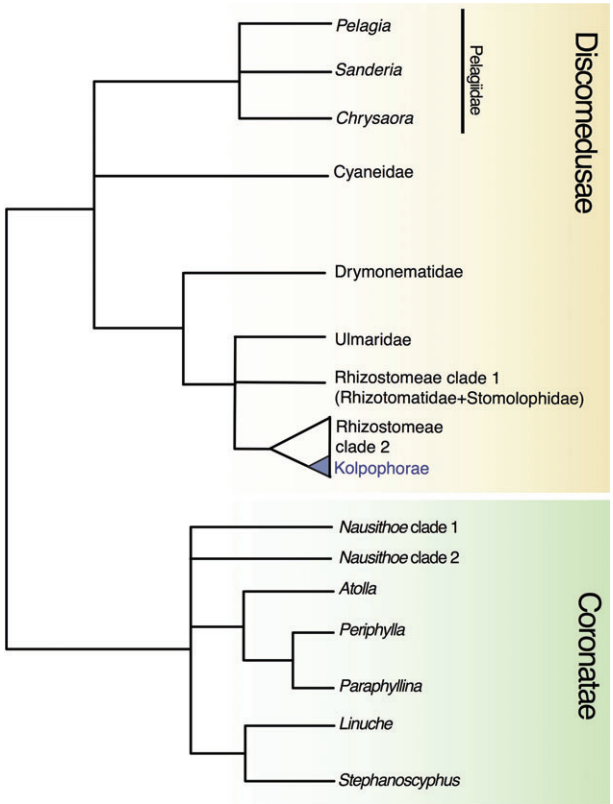

Fig. 1. Phylogenetic hypothesis of scyphozoan relationships based on Bayha *et al.* (2010). Scyphozoa is composed of two major clades, the Coronatae (green) and the Discomedusae (gold). Coronatae contains the paraphyletic genus *Nausithoe*, the clade containing *Atolla*, *Periphylla* and *Paraphyllina*, and a clade containing *Linuche* and *Stephanoscyphus*. Within the Discomedusae there are at least three major lineages: (1) Pelagiidae including all known *Chrysaora*, *Pelagia*, and *Sanderia*; (2) Cyaneidae including the genera *Cyanea* and *Desmonema*; and (3) a currently unnamed clade composed of several major groups: Drymonematidae, Ulmaridae and the Rhizostomeae clades 1 and 2 (the latter containing the monophyletic group Kolpophorae).

the bells are sometimes ornately textured), and in species with marginal tentacles, the tentacles trail gently behind the animal.

There are two Linnaean orders within Discomedusae, the Rhizostomeae and Semaeostomeae (Daly *et al.*, 2007), which are supported by cladistic analysis (Marques & Collins, 2004), but not by multiple studies using molecular data, from which it appears that Semaeostomeae is paraphyletic with Rhizostomeae (Collins, 2002; Bayha *et al.*, 2010) (Fig. 1). The semaeostomid Pelagiidae species form a monophyletic group, but the Ulmaridae (including moon jellies) form a clade with 'true Rhizostomeae' (Bayha *et al.*, 2010), rather than being affiliated with Pelagiidae. Fig. 1 presents the evolutionary relationships within Scyphozoa outlined by Bayha *et al.* (2010). This phylogeny (using 18S and 28S rDNA) is the most complete phylogeny of Scyphozoa to date, with full taxon sampling at the family level. Evolutionary hypotheses generated using molecular data provide us with an opportunity to map developmental and life-history characters onto a phylogenetic framework. We can thus begin to retrace the evolutionary history of key features in scyphozoan life cycles.

III. ANATOMY OF STROBILATION AND MEDUSA MORPHOGENESIS

Historically, each life-cycle stage was described as separate species (reviewed in Russell, 1970). It is from these original descriptions that we derive the terms for each stage: 'scyphistoma', 'strobila', and 'ephyra'. The polyp and strobila stages were first described by Sars (1829) and given the species names *Scyphistoma filicorne* and *Strobila octoradiata*, respectively. Eschscholtz (1829) described a small star-like medusa, for which he created the genus *Ephyra*. It was not until the process of strobilation was first documented by Sars (1835) that the scyphistoma, strobila, and ephyra were brought together into a single life cycle.

Sars' original description of a strobila was that of a polydisc strobilator. Polydisc strobilators produce many ephyrae per polyp, resembling their namesake 'strobilus' – the reproductive 'pinecone' of a fir tree. However, this is one of two possible forms of strobilation. Some species are monodisc strobilators – forming only one ephyra during strobilation (Fig. 3). Monodisc strobilation can be thought of, in some respects, as polydisc strobilation with only the topmost ephyra being produced. Regardless of the number of ephyrae produced per strobilation event, the earliest signs of strobilation appear evolutionarily conserved.

(1) Early signs of strobilation

In the monodisc strobilator *Cassiopea xamachana*, Bigelow (1892) reported one of the earliest visible signs of strobilation to be conical lobe outgrowths at the base of polyp tentacles. I have also observed this in the monodisc strobilators *Mastigias papua*, *Cephea* sp. and *Cotylorhiza tuberculata*. In the polydisc strobilator *Aurelia aurita* from Norfolk, VA, USA, 24 h after strobilation induction, Spangenberg (1991) observed that the base of interradial and perradial polyp tentacles broadened, and this continued for up to 48 h at 28°C. Calder (1982) observed this thickening 6 h prior to formation of the first strobilation segment in *Stomolophus meleagris*. In all cases these broadening tentacles are at the site of future rhopalia (sensory organ) development (See Section III.3*a*). Collectively, all these species are in the clade containing Ulmaridae + Rhizostomeae, so this may represent a synapomorphy for this clade, or possibly for Discomedusae or Scyphozoa, although additional species from the Pelagiidae, Cyaneidae, and Coronatae will need to be examined.

For the Pelagiidae species *Chrysaora chesapeakei* (formerly know as *Chrysaora quinquecirrha* (Bayha, Collins & Gaffney, 2017)), the first evidence of polydisc strobilation is reportedly elongation of the body column directly below the polyp

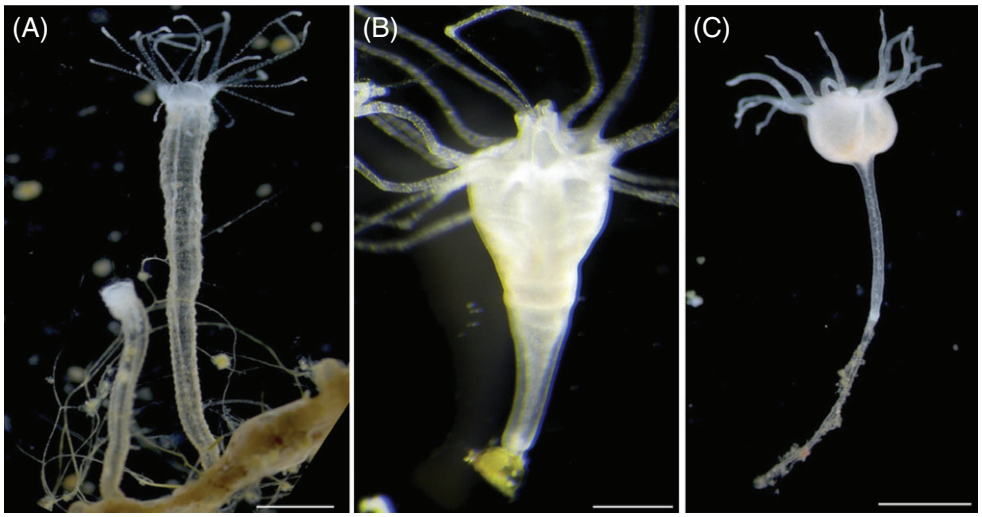

Fig. 2. General polyp anatomies for different scyphozoans. All scyphozoan polyps have columnar bodies that attach to a hard substrate at the aboral surface. The oral surface is composed of a ring of tentacles surrounding a central mouth, collectively known as the 'oral disc'. All polyps have cord muscles, which they use in retraction. (A) *Linuche* sp. coronate polyps live within chitinous tubes, into which they contract when startled. (B, C) Discomedusae polyps can be found in one of two general morphologies: (B) a polyp without a tube (*Chrysaora chesapeakei*); or (C) a polyp with a small tube encasing all or part of the aboral stalk (*Mastigias papua*). Scale bars: 1 mm.

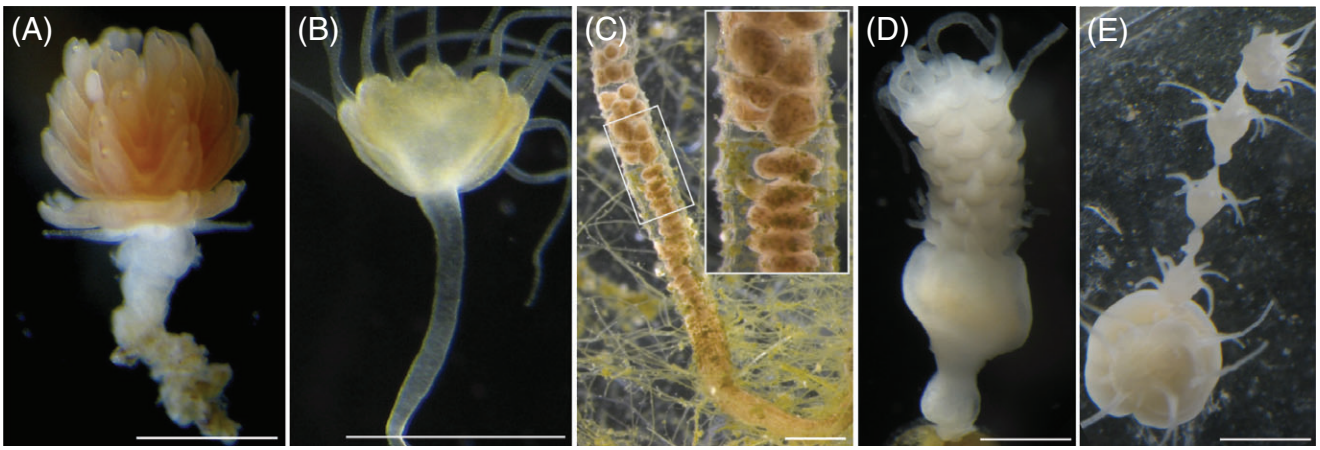

Fig. 3. Variation in scyphozoan strobilation. (A) *Aurelia* sp. polydisc strobilation occurs when one polyp produces many ephyrae in succession (see Fig. 4). (B) *Phyllorhiza punctata*. Monodisc strobilation occurs when only one ephyra is produced per polyp. (C) In the coronate *Linuche* sp. polyps can produce planuloids *via* strobilation (pictured), or ephyrae (not shown). (D, E) Some scyphopolyps can produce further polyps *via* strobilation, as exhibited by this unidentified discomedusan polyp. Scale bars: 1 mm.

tentacle ring; this transient morphological feature, termed the 'neck', forms during the 'neck-formation stage' (Loeb, 1974*a*). According to Russell (1970), this elongation is due to cell flattening rather than cell proliferation. Chapman (1966) also reported lengthening in *Aurelia* sp. polyps, although Spangenberg (1968) found no evidence of lengthening in a different *Aurelia* line. Slight elongation has also been observed in *S. meleagris* (Calder, 1982). This neck will ultimately become the first tissue fold in polydisc strobilation (see Section III.2). These observations are from two of the three major lineages in Discomedusae (see Fig. 1; Pelagiidae, and Rhizostomae + Ulmaridae), suggesting that polyp neck formation may be a conserved feature of Discomedusae.

(2) Segmentation formation

The most unambiguous sign of strobilation initiation is the formation of a strobilation furrow directly under the polyp tentacle whorl (Fig. 4A); this is the beginning of the segmentation phase of polydisc strobilation, and segregation of future ephyra tissue in monodisc strobilation. This furrow seperates the topmost part of the polyp calyx and oral disc (which are destined to become the topmost, or 'terminal' ephyra) from the main body column. In polydisc strobilation, once the segmentation process has begun, additional furrows are added at equal distances underneath the first furrow (Fig. 4B, C), or roughly 0.18 mm apart in the moon jelly *A. aurita* (Kroiher, Siefker & Berking, 2000). According to

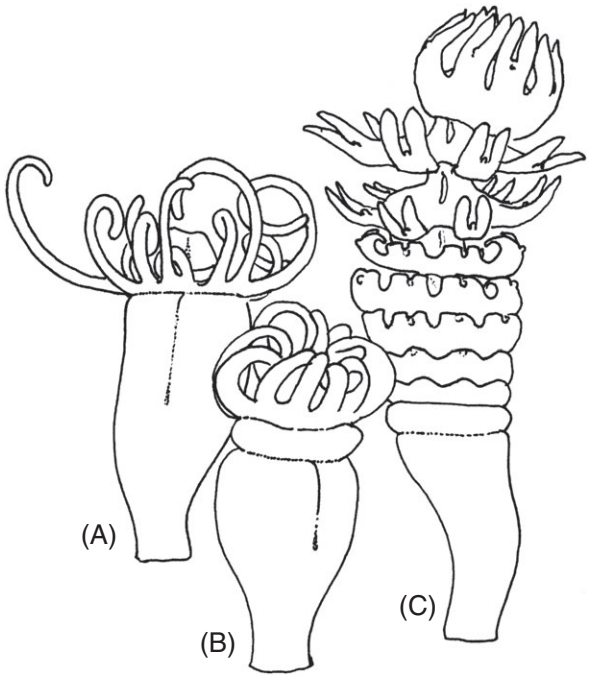

Fig. 4. The process of segmentation and ephyra formation in a Discomedusae polydisc strobilator. (A) One of the first signs of strobilation is a furrow or constriction that forms directly under the polyp tentacles. (B, C) Additional furrows form sequentially along the polyp body, equidistant apart. (C) As strobilation progresses, ephyra rudiments develop sensory and muscle structures, eventually begin pulsing, and ultimately break free of the strobila. The processes of strobilation ends when polyp tentacles reform at the base of the strobila. All ephyrae will eventually detach, leaving a small polyp behind.

Berrill (1949), these grooves undergo cellular proliferation and heightening in both the epidermis and gastrodermis, and the discs of tissue between each furrow are subject to cell proliferation in the ecto- and endoderm (Russell, 1970). The extracellular matrix between the ecto- and endoderm, known as 'mesoglea', is thin in ephyrae discs, with fibres randomly oriented, while in the constrictions it is thicker and fibres are branched (Bynum & Black, 1974). In *A. aurita*, ephyra disc size appears unrelated to polyp size, and polyps of different sizes often produce similar-sized ephyrae, although the number of segments is generally much greater in larger polyps than in smaller polyps (Kroiher *et al.*, 2000). In the monodisc strobilator *Ca. xamachana,* a furrow forms at the base of the future ephyra (Bigelow, 1892), and I have also observed this furrow in *Co. tuberculata*. Whether after the formation of one ephyra or many, segmentation and furrow formation eventually cease, and the unsegmented base of the polyp regrows tentacles and a mouth. This newly regenerated polyp can eat, grow, and even strobilate again in the future.

Segmentation is an integral part of strobilation. In most cases, each segment becomes a developing ephyra, termed 'ephyra rudiment'. However, the process of segmentation precedes ephyra formation, and some authors consider segmentation and ephyra development to be two distinct (although interrelated) developmental events (Berrill, 1949; see review in Spangenberg, 1968). This is supported by the fact that segments do not always become ephyrae. For example, *Aurelia* sp. (and *Ch. chesapeakei*, personal observations) strobilae exposed to stress can become 'polyp-strobilae', where each segment develops into a polyp (see review in Spangenberg, 1968) (Fig. 3D, E). Strobilae can also produce planula-like 'planuloids,' in addition to or instead of ephyrae (Fig. 3C). Variation in the plasticity of segmentation, and the ability of strobilae to form polyps, planuloids, or ephyrae, is not well understood. Although these non-canonical strobilae may harbour clues to understanding the developmental processes associated with strobilation, they are also somewhat rare, with most strobilae producing ephyrae.

(3) General anatomy and development

Once segments are fated to become ephyrae, the development of ephyral features depends on the location of the segment. In monodisc strobilators and in the topmost ephyra of polydisc strobilators, the ephyra forms from the upper calyx and oral disc of the polyp, while the remaining segments in polydisc strobilators form from tissue segments in the calyx. For polydisc strobilators, this results in developmental differences between the topmost and lower segments. For example, the topmost ephyrae forms a mouth at the same location as the polyp mouth, while lower segments must form mouths *de novo*. Below I outline the general development of ephyrae, followed by sections on the differences in ephyral development for upper and lower discs (see Sections III.4 & 5).

(*a*) *Rhopalia*

Rhopalia are finger-shaped sensory structures at the bell margin of ephyrae and medusae. Although rhopalia share the same general shape and location across Scyphozoa, the morphology of mature rhopalia is variable (Arai, 1997). For example, the rhopalia of many species bear rudimentary pigment spots that may serve as light sensors, but in *Paraphyllina intermedia*, rhopalia have lenses similar to those found in box jellyfish, and may be used for sophisticated vision. Because rhopalia are variable in their morphology across species, the development of rhopalia is also variable. However, rhopalum development is only known from a few species, of which the best studied is *Aurelia* sp. (Hyman, 1940; Arai, 1997; Nakanishi & Hartenstein, 2009).

In order to understand rhopalial development in *Aurelia* sp., it is first necessary to understand the anatomy of rhopalia in adult medusae of this species. The rhopalium can be thought of as a finger-like projection. The inside, or palm side, of this finger faces down, in the direction of the oral surface, while the topside of the finger faces up, towards the aboral surface. Schafer (1878) described three segments in *A. aurita* rhopalia, demarcated by small creases, which are analogous to the three segments of a

finger: a 'terminal segment' analogous to the finger-tip; an intermediate segment, analogous to the mid-segment of the finger; and a basal segment, analogous to the finger segment closest to the hand. On the tip there is a cluster of large, crystal (statolith)-containing endodermal cells, termed 'lithocytes', which together form the 'statocyst', a large mass of heavy crystal-bearing cells. The weight of this statocyst bends the finger-like rhopalium in response to gravity. On the top (aboral) side of the rhopalium and proximal to the statocyst, bridging the terminal segment and intermediate segment (like a knuckle), there is an epidermal pigmented spot, termed the 'pigment-spot ocellus', which is likely sensory; it is not clear if it is light sensory, chemosensory, or some combination thereof (Nakanishi & Hartenstein, 2009). Proximal to the pigment-spot ocellus in the intermediate segment, there is a thickened plate of ciliated epidermal cells, termed the 'touch plate', which may be involved in sensing tilt (Chapman & James, 1973). Proximal to the touch plate the bell and rhopalium meet. Moving to the oral side of the rhopalium, or the palm side of our finger analogy, proximal to the statocyst and between the terminal and intermediate segments, there is a cluster of endodermal pigmented cells overlaid by ectodermal photoreceptor cells, which together form the 'pigment-cup ocellus', situated opposite the pigment-spot ocellus (in a similar fashion to a joint crease sited opposite the knuckle). The pigment-cup ocellus may be involved in light sensing (Terufumi & Yoshida, 1973). Combined, these four structures – the statocyst, pigment-spot ocellus, touch plate, and pigment-cup ocellus – comprise the major sensory components of the rhopalium.

Each segment and sensory structure within the rhopalium has its own population of neurons, each with unique features (Nakanishi & Hartenstein, 2009). At least two populations of neurons connect the rhopalium to the main medusa body: (1) neurons that can be stained using an anti-tyrosinated tubulin antibody, whose neurites leave the rhopalium and join the large motor neuron network (see Section III.3*b*), and (2) neurons that can be stained by anti-FMRFamide, whose neurites leave the rhopalium and join the diffuse nerve net (see Section III.3*b*) (Nakanishi & Hartenstein, 2009). Nakanishi & Hartenstein (2009) identified at least seven sensory cell groups with neuronal processes.

Above the rhopalium on the aboral surface (equivalent to the top of the hand), there is a tissue projection, termed a 'hood'. When the animal swims oral-side up, the statocyst bends the rhopalium down and a presumably mechanosensitive, ciliated surface of the touch plate comes into contact with the hood. This represents one mechanism by which scyphomedusae may sense direction. Schafer (1878) also described two sensory pits proximal to the rhopalium, one on the aboral surface (termed the 'fovea nervosa superior') and one on the oral surface hood (termed the 'fovea nervosa inferior'), although their exact function is not known.

Ultimately sensory information from the rhopalia is transmitted to the nervous system. Combined, the rhopalia and nervous system coordinate the behaviour and responses to the environment in ephyrae and medusae.

(*b*) *Nervous system and muscles*

The polyp, strobila, and ephyra nervous systems are temporally linked. For this reason, understanding the origin of the ephyra nervous system can only be done in the context of polyp and strobila nervous systems. The polyp nervous system of *A. aurita* is reported to be exclusively ectodermal (Chia, Amerongen & Peteya, 1984). Two types of neurons have been identified – epithelial sensory cells in the tentacles, and sub-epithelial neurons in the tentacles, oral disc, and muscle cord. Anti-FMRFamide-positive neurons are present in the polyp oral disc and tentacles (Nakanishi *et al.*, 2008). However, neurons have not been found in regions of the body where muscle is absent (Chia *et al.*, 1984). Portions of the body column that do not contain muscle do not respond to mechanical stimulation in *A. aurita* (Chia *et al.*, 1984) or *Ch. chesapeakei* (Loeb & Hayes, 1981). In *A. aurita* from Woods Hole, MA, USA, electrical potentials were also not detected in the body column (Schwab, 1977*a*). *Chrysaora chesapeakei* is within the clade Pelagiidae, while *A. aurita* is within the Rhizostomeae + Ulmaridae clade. Thus, innervation of polyp musculature but not the surrounding body column may be a shared feature of discomedusan polyps.

Polyp neurons may be involved in strobilation induction or progression, and integration of ephyrae neuronal responses in the strobila. During early and mid-strobilation, polyp neurons in *Ch. chesapeakei* secrete substances into the surrounding tissue (Section VII). In later-stage polydisc strobilae, ephyrae beat independently while still attached, suggesting that each ephyra has an autonomous nervous system. However, Horridge (1956) noted that touching the base of an *A. aurita* strobila with a seeker initiated a wave of ephyra spasms along the strobila, with each ephyra contracting in succession. By contrast, Schwab (1977*a*) could not induce full-strobila contraction in *A. aurita* by stimulating the strobila base, but did achieve this by stimulating the topmost ephyra. These results suggest that ephyrae remain neuronally integrated during strobilation, likely through neurons associated with the 4–6 muscles that run from the oral disc to the foot, termed the polyp 'cord muscles'. While work on coordinated ephyral behaviour is limited to *A. aurita*, the shared morphology between *A. aurita* and *Ch. chesapeakei*, particularly with regard to muscle-associated neurons, suggests that a coordinated strobila response may also exist in pelagiid scyphozoans.

In contrast to polyps, which only have a nerve net, ephyrae of *Aurelia* sp. have at least two major components to their nervous system. One population of neurons coordinates pulsation during normal swimming (Horridge, 1956; Satterlie & Eichinger, 2014). These swimming-associated neurons are large, mostly located in proximity to swimming muscles, correlate with strong anti-tubulin antibody staining (Satterlie & Eichinger, 2014), and originate at the marginal ganglia associated with rhopalia (Horridge, 1956). Cutting medusae at the bell margin severs the large neurons associated with musculature and disrupts coordinated pulsing (Romanes, 1876). By contrast, the feeding response and

spasm response (where the ephyra curls into a ball that is maintained for longer than a single beat) are coordinated by the diffuse nerve net (Horridge, 1956). This diffuse nerve net consists of a web of smaller cells over the body, and shows strong anti-FMRFamide staining (Satterlie & Eichinger, 2014).

The large swimming-associated neurons likely develop *de novo*, as their functional outputs are not similar to any known polyp-like behaviours (Schwab, 1977*a*,*b*). By contrast, polyp contraction and ephyra spasms show more similarities, as do polyp and ephyra feeding responses (Schwab, 1977*a*,*b*). Schwab (1977*b*) suggests that swimming-associated neurons develop during strobilation and are superimposed over a polyp-like diffuse nerve net. This is supported by the presence of anti-FMRFamide-positive neurons in both the polyp oral disc (Nakanishi *et al.*, 2008) and ephyra diffuse nerve net (Satterlie & Eichinger, 2014). Thus, there may be some continuity between the polyp and ephyra nervous systems, at least for the topmost segment. However, this has not been examined developmentally. It is possible that these analogous nerve nets are not, in fact, developmentally homologous. For example, the earliest larval stage – the planula – also has anti-FMRFamide-positive neurons, which degrade and re-form during metamorphosis into a polyp (Nakanishi *et al.*, 2008). Degradation and re-formation of similar neuron types is an alternative hypothesis to the formation of the ephyra neurons *via* the persistence of polyp neurons.

Sensory and neuronal physiologies ultimately result in ephyra behaviour through the action of ephyra 'muscles'. In adult *Cyanea capillata,* muscle-associated neurons weave through myoepithelial cells that form striated swimming muscle (Anderson & Schwab, 1981). In ephyrae, musculature can be categorized into three parts, with the whole muscle system appearing like a cartoon drawing of the sun: (*i*) a band of striated circular muscle forming a ring around the ephyra margin, (*ii*) rays of striated radial muscle extending from this ring into the swimming arms, each terminating near the tip of a rhopalial lappet (two rays per swimming arm), and (3) non-striated myoepithelial cells radiating from each of the four corners of the ephyra manubrium (Helm *et al.*, 2015).

Further studies on the neuroanatomical and neuromuscular changes that take place during strobilation are needed. This is particularly true for monodisc strobilators, as well as a broader diversity of polydisc strobilators.

(*c*) *Gastrovascular system*

The ephyra gastrovascular system (GVS) forms in the same location as the polyp GVS, and may have similar features (such as an absence of neurons), although it is unclear if ephyra gastrodermal cells replace the original polyp cells during metamorphosis, or if the polyp gastrodermis is remodelled to form the ephyra gastroderm.

In liberated ephyrae, the stomach is located in the middle of the ephyra. Rhopalial canals radiate off the stomach and into each ephyra arm, with a velar canal between each arm. This gives the GVS a 'star-like' appearance in all Scyphozoa (Straehler-Pohl & Jarms, 2010). The developmental pattern of the GVS, both during ephyra formation and as ephyrae mature, may be an important evolutionary character: GVS development in liberated ephyrae is used as a morphological character to distinguish between the two suborders Kolpophorae and Dactyliophorae [see Holst *et al.*, 2007; it should be noted, however, that Dactyliophorae may be paraphyletic with respect to Kolpophorae (Bayha *et al.*, 2010)]. Further studies on GVS development in Scyphozoa may lead to new evolutionary insights.

(4) Metamorphosis of the topmost segment

For the vast majority of ephyra-producing polydisc strobilae, the topmost segment forms from the polyp upper calyx and oral disc, while the remaining rudiments form from body column tissue. Therefore, the topmost ephyra develops in a slightly different manner compared to all other segments, representing a fascinating example of two different developmental starting points – polyp calyx and body column segments – resulting in the same developmental outcome: the ephyra.

One of the first signs of strobilation for some species is the thickening of polyp tentacles (Section III.1), and these same thickened tentacle bases give rise to rhopalia. Spangenberg (1991) provided a thorough overview of rhopalia development at the base of polyp tentacles for an *A. aurita* line from Norfolk, VA, USA. Prior to the initiation of strobilation, numerous long 'polyp-type' kinocilia are found on polyp tentacles at the location where rhopalia will form. As a rhopalium develops, these 'polyp-type' kinocilia are largely replaced by shorter, ephyra-type kinocilia. Developing rhopalia form small knobs protruding from the swollen base of the polyp tentacle. These rhopalia knobs are at a slight angle, facing towards the polyp mouth. At the same time, polyp tentacles decrease in size. Seventy-two hours post induction at 28°C, the newly formed rhopalia at the base of tentacles are rather bulbous, and the polyp tentacles are nearly fully resorbed. The rhopalium changes shape throughout development in association with the formation of different sensory structures. When the ephyrae begin to pulse weakly, the rhopalia appear thinner and–because rhopalia are necessary for normal pulsation in medusae – are likely functional (Schwab, 1977*a*; Spangenberg, 1991). Eventually the topmost ephyra rudiment breaks free of the strobila stack to begin life as the first in a series of liberated ephyra. The formation of rhopalia at the tentacle base, and the absorption of tentacles, is reported in both Rhizostomeae + Ulmaridae and Pelagiidae, and is likely a shared feature within Discomedusae. Additional research on coronates in needed (see below).

Rhopalial development is closely linked to the formation of pairs of rhopalial lappets, and together these structures – two lappets with an intervening rhopalium – are termed 'rhopalar arms' (Nakanishi & Hartenstein, 2009). In the monodisc strobilator *Ca. xamachana*, lappets form on either side of the rhopalium-bearing tentacle as the tentacle is being resorbed (Bigelow, 1892). This is also true in *A. aurita* (Spangenberg, 1991). This general trend may be true across

Discomedusae: rhopalia form at the base of polyp tentacles, and rhopalia lappets form to the side. Additional observations in Pelagiiade and Cyaneidae are needed.

Rhopalia are part of the ephyra neuromuscular system, and several studies have examined the neuromuscular anatomy and physiology of the polyp calyx and first ephyra, although only from *A. aurita*. In the tentacles of *A. aurita* from San Juan Island, WA, USA, neurons form a plexus at the tentacle base, and spread into the oral disc. However, they do not form a well-defined nerve ring (Chia *et al.*, 1984). Schwab (1977*a*), studying polyp from Woods Hole, MA, USA, recorded electrical potentials at the site of rhopalia formation at the base of polyp tentacles before and during strobilation. In early strobila, there was no difference in the pattern of electrical potentials in polyps and these young ephyrae. Once tentacles are absorbed, there is a period of quiescence before medusa-like electrical potentials are detected (Schwab, 1977*a*). In medusae, all medusa ganglion potentials, i.e. potentials associated with pulsing, are transmitted between rhopalia, which coordinate movement. However, in early developing ephyrae, this is not necessarily the case. Medusa ganglion potentials are seen in early rhopalia, but are not linked between them (Schwab, 1977*a*).

In contrast to these developmental processes of the topmost segment in the discomedusan strobila, the fate of the topmost segment in coronates is considerably more variable. The calyx of coronate polyps can be distinguished from discomedusan polyps in several ways, most prominently by the presence of a ring sinus (Chapman & Werner, 1972). This difference may be correlated with differences in the fate of the topmost segment between Coronatae and Discomedusae. Werner (1973) notes that, at the start of strobilation in several coronate species, the polyp oral surface is reduced to form a tissue layer or plug, which may secrete a periderm operculum (although not in *Nausithoe punctata* and *N. racemosus*), sealing the tube during strobilation; when ephyrae are mature enough to escape the tube, this plug degrades and is cast off. However, Werner (1973) also reports that, in species he refers to as *Stephanoscyphus spec*. 7, and *Stephanoscyphus komaii*, the cellular operculum does not detach and is instead resorbed by the topmost ephyra. By contrast, Silveira & Morandini (1998) observed that strobilating *Linuche unguiculata* could, but did not always, form a peridermal operculum, and did not form tissue plugs. In five out of six specimens of *L. unguiculata*, the segment containing the oral disc formed an ephyra, while more-aboral discs formed planuloids. In the sixth specimen, only planuloids were formed (Silveira & Morandini, 1998). From the available information, the fate of the polyp oral disc appears more variable in coronates than in discomedusans.

(5) Metamorphosis of lower segments

In polydisc strobilators, ephyrae below the topmost segment form from polyp body column tissue, rather than the polyp calyx; for this reason the development of lower segments is slightly different from the topmost segment, even if the morphogenic results are ultimately the same. Rhopalia and rhopalial lappets on the topmost segment of discomedusans form in association with polyp tentacles. In lower segments these structures form *via* 'arm buds' (Helm *et al.*, 2015). Arm buds first appear as small protrusions on an otherwise circular ephyra rudiment. These arm buds are arranged equidistantly, oriented away from the central body column, and are sometimes pointed. Arm buds on lower segments are present across a range of discomedusan scyphozoans [e.g. *Ch. chesapeakei* and *Ch. achlyos* (Helm *et al.*, 2015), *S. meleagris* (Calder, 1982), and *Rhizostoma octopus* (Holst *et al.*, 2007)]. In the coronate *Nausithoe maculate*, slight protrusions in the margin of early ephyrae also appear to be arm-bud like (Eggers & Jarms, 2007). However, there is no clear evidence for arm buds in the coronate *Atorella vanhoeffeni*; rhopalia and rhopalial lappets appear to develop distinctly, rather than as part of a single arm bud (Eggers & Jarms, 2007). The formation of armbuds in lower segments of polydisc strobilators appears conserved in Discomedusae, and may be present across Scyphozoa, although examination of more coronates in needed to determine whether the lack of arm buds in *At. vanhoeffeni* is a derived or ancestral state for Coronatae.

For animals with arm buds, the future rhopalium forms at the tip; this can be seen in *Ch. chesapeakei* using confocal microscopy (Fig. 5). In this species, approximately midway in time between segment formation and ephyra liberation, rhopalia become clearly visible to the naked eye as small nodules. At a comparable stage in *Aurelia* sp. 1, small statoliths are also present in the rhopalia (Nakanishi & Hartenstein, 2009).

To better characterize the timing of rhopalial development, Nakanishi & Hartenstein (2009) investigated the sensory and nervous system development of *Aurelia* sp. 1 and defined five different stages. The first stage begins approximately midway in ephyra development, with small lappets and radial muscle having already formed on either side of the rhopalium (although they are less than 100 μm long). The statolith begins forming at this stage, and some neurons (including diffuse nerve net neurons) have formed, but not all neuron types are present. In the second stage, both the lappets and rhopalia are elongate, the three rhopalium segments are present, and many of the rhopalia-specific neuron populations are present, except for select sensory cells, including those associated with the ocelli, which have not yet formed. At the third stage, which may be applicable to both the lower and topmost segments, the animal begins pulsing, indicating that the pacemaker function of rhopalia is active, and anatomical connections between the rhopalia nervous system and the rest of the developing ephyra nervous system are evident. During the second and third stages, the touch plate cells develop [although Spangenberg, 1991 asserted that the touch plate does not develop until after ephyra liberation]. The fourth stage is the liberated ephyra, at which point the pigment-cup ocellus begins to develop. Nakanishi & Hartenstein (2009) label the fifth stage as the metaphyra – a transition between classic ephyra

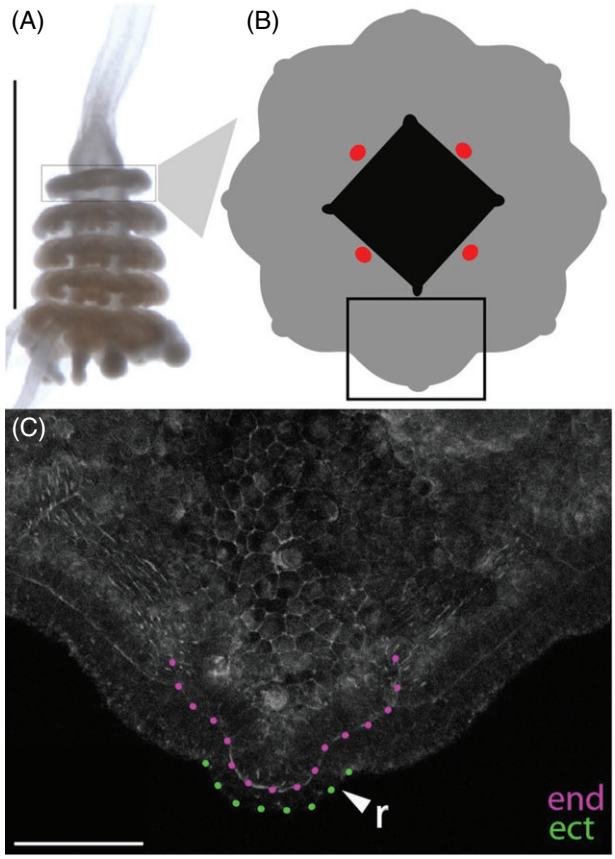

Fig. 5. Young ephyra rhopalium in *Chrysaora chesapeakei*. (A) A strobila stack with the grey rectangle indicating a young ephyra rudiment. (B) Diagram of the same rudiment, viewed orally, with polyp cord muscle in red, and a box outlining the approximate location of C. (C) Confocal microscopy image of a developing arm bud, stained with BODIPY phallacidin. The developing rhopalium (r) is indicated. The dotted purple line indicates the border of the endoderm folding inward at the margins; the dotted green line indicates the outer surface of the ectoderm. Scale bars: A = 1 mm C = 50 μm.

and medusa morphologies – at which time the pigment-spot ocellus differentiates. This timeline established by Nakanishi & Hartenstein (2009) provides an excellent framework for comparing rhopalia development across Scyphozoa, including the development of unique rhopalial features such as lenses in *P. intermedia*.

While the rhopalia are developing, small tissue protrusions on either side of the future rhopalium form and differentiate into the rhopalial lappets in various members of Scyphozoa [e.g. *Ch. chesapeakei* and *Ch. achlyos* (Helm *et al.*, 2015), *S. meleagris* (Calder, 1982), *R. octopus* (Holst *et al.*, 2007), *N. maculate*]. This is true regardless of whether or not the rhopalial lappets form on an arm bud [e.g. *At. vanhoeffeni* (Eggers & Jarms, 2007)]. The presence of rhopalial lappets is a synapomorphy of Scyphozoa, and rhopalial lappets are used by ephyrae for swimming (Feitl *et al.*, 2009) and to manoeuvre food to the mouth, which they achieve with a system of muscle groups.

The stages of ephyra muscle formation in the lower segments were identified using actin staining in *Ch. chesapeakei* and *Ch. achlyos* (and in the direct-developer *Pelagia noctiluca*) (Helm *et al.*, 2015). Actin-rich clusters appear first in the epidermis of early arm buds at the site of future circular muscle. As development of an arm bud progresses, these actin-rich clusters become more numerous and populate the site of future radial muscle. At around the same time that noticeable ephyra pulsation begins, these clusters show evidence of striation, eventually forming circular and striated muscle bands (Helm *et al.*, 2015). Helm *et al.* (2015) found no evidence of polyp muscle being remodelled to form ephyra musculature. This suggests that ephyra musculature forms *de novo* during strobilation. Ephyra musculature is in close proximity to the gastrodermis, with circular muscle forming a ring around the main gastrovascular cavity, and radial muscle present on either side of the rhopalial gastrovascular canals.

The opening to the gastrovascular system – the mouth – must form from different tissue types in the topmost and lower segments. In the topmost segment, the ephyra mouth forms at the site of the polyp mouth, but in the lower segments the ephyra mouth must develop at the site of the tissue constriction that forms between rudiments. In *Ch. chesapeakei*, the ephyra mouth takes on a four-point star shape, with the four polyp cord muscles running through the indentations of the future mouth (Helm *et al.*, 2015). Because each ephyra is linked to the ephyra above *via* a mouth-to-exumbrella connection, completion of mouth development may be associated with strobila fracture and ephyra liberation.

Concurrent with the development of ephyra structures from ephyra rudiments, polyp structures are being reduced. In *Aurelia* sp. polyp stinging cells known as 'atrichous polyspiras nematocysts', degrade during ephyra formation (Spangenberg, 1968). Polyp cord muscle moves inwards towards the centre of the strobila and becomes thinner (Eggers & Jarms, 2007; Helm *et al.*, 2015). The remnant of this polyp muscle runs through the ephyrae manubria, and is among the last tissue connecting one ephyra rudiment to the next in the discomedusan *Ch. chesapeakei* (Helm *et al.*, 2015) and in several coronates (Eggers & Jarms, 2007). In liberated ephyrae of *A. aurita* and *Ch. chesapeakei*, all polyp cord muscle degrades, and the location of past cord muscle becomes the site of future gastric filaments in the ephyra gastrodermis and subumbrella epidermis (Chuin, 1930; Russell, 1970).

IV. PHYLOGENETIC VARIATION IN STROBILATION TYPE

There are two distinct types of strobilation in Scyphozoa: polydisc (where multiple ephyrae are produced) and monodisc (producing only a single ephyra). Initial phylogenetic analyses suggested that monodisc strobilation was specific to Rhizostomeae (Collins, 2002), and arose from ancestral polydisc strobilation (Marques & Collins,

2004). However, Semaeostomeae is likely paraphyletic with Rhizostomeae (Bayha *et al.*, 2010), and not all animals within this Rhizostomeae + Ulmaridae clade exhibit monodisc strobilation. Fig. 6 shows the distribution of mono- and polydisc strobilation for select scyphozoans. Character states for most species were derived from the literature supplemented with personal observations, and references are provided in Table 1.

Polydisc strobilation is more broadly distributed than monodisc strobilation, and has been hypothesized as the ancestral condition (Collins, 2002) (Fig. 6). The most parsimonious explanation for the origin of monodisc strobilation is that it arose independently at least twice. The vast majority of monodisc strobilators are rhizostomes in the taxonomic suborder Kolpophorae (Kramp, 1961), which is also strongly supported in molecular phylogenies based on 18S and 28S ribosomal DNA sequences (Bayha *et al.*, 2010). Monodisc strobilation as a synapomorphy of Kolpophorae is in agreement with previous observations (Holst *et al.*, 2007; Dawson & Hamner, 2009). For species in the clade Kolpophorae where the full life cycle is known, polyps have a relatively small cup-shaped calyx and a long narrow peduncle (Holst *et al.*, 2007) (Fig. 2C). This small calyx physically restricts the amount of tissue that can metamorphose into ephyra rudiments (Berrill, 1949). Sporadic monodisc strobilation is sometimes observed in polydisc strobilators when polyps are very small (Spangenberg, 1968), suggesting that calyx size may be an important variable related to ephyra production. Thus, monodisc strobilation in Kolpophorae may be related to their unique polyp anatomy (Holst *et al.*, 2007).

Monodisc strobilation has also been documented in *Sanderia malayensis* (Uchida & Sugiura, 1978; Adler & Jarms, 2009). *Sanderia malayensis* is nested within Pelagiidae (Bayha *et al.*, 2010), and all other species in this clade that strobilate are polydisc strobilators (Fig. 6). However, monodisc strobilation is not the only form of post-embryonic development that sets *Sa. malayensis* apart from its close relatives in Pelagiidae. *Sanderia malayensis* polyp asexual reproduction is incredibly diverse, leading Adler & Jarms (2009) to create several new categories of asexual reproduction just for this species (including budding directly off the gastrodermis). Whether this diverse asexual reproduction has any connection with its unique mode of strobilation is not known.

V. LIFE-CYCLE VARIATION AND OTHER FORMS OF MEDUSA DEVELOPMENT

Although strobilation is the most common mechanism by which medusae are produced, several scyphozoans have evolved radically altered life histories, where strobilation has either been reduced or lost completely. Diverse forms of direct development may have originated independently as many as four times within Scyphozoa (Fig. 7), and at least once facultatively. In the obligate direct-developing scyphozoans *Pelagia noctiluca*, *Periphylla periphylla*, *Poralia* sp. (possibly) and *Stygiomedusa fabulosa*, the planula and/or polyp stages are either absent or highly modified (Table 1). *Pelagia noctiluca* and *Per. periphylla* both develop medusae without an apparent polyp stage; these life cycles were first placed into an evolutionary context by Dawson & Hamner (2009). However, beyond the absence of a polyp in these two species, the process of direct development is markedly different.

Of all direct-developing medusae, *Per. periphylla* is the most extreme, having lost the planula, polyp and ephyra stages. *Periphylla periphylla* is a coronate with a deep- and cold-water distribution; it is the only coronate known to have direct medusa development (Jarms *et al.*, 1999). Jarms *et al.* (1999) collected young *Per. periphylla* from plankton tows in Lurefjorden, Norway, and catalogued eight distinct developmental stages. In stages 1–4, extremely large (1.6 mm), neutrally buoyant eggs develop not into planulae, as in all other scyphozoans, but into thimble-like immature medusae (Fig. 8Ai, ii). At stage 5, 16 developing lappets and four rhopalia are visible on the margin, although the mouth has yet to open (Fig. 8Aiii). The marginal lappets and rhopalia are similar in appearance to ephyrae, but the apex of the bell is quite bulbous, differing from the flat morphology of other ephyrae. Slightly later (stage 6), tentacle buds appear and the mouth opens. In stage 7, all the features of young medusae are present (12 tentacles, rhopalia; Fig. 8Aiv), and in stage 8, medusae become pigmented. Development takes 2–3 months from fertilization to feeding medusae, and light exposure causes pigment loss and lethality (Jarms, Tiemann & Båmstedt, 2002). For this reason, only stages 1–4 were reared in captivity (Jarms *et al.*, 2002). Whether the life cycle of *Per. periphylla* is unique is not clear. There are many deep and/or cold-water coronates that remain poorly known.

Pelagia noctiluca is a globally distributed species within Pelagiidae. Unlike *Per. periphylla*, for which direct development was only recently described, direct development of *Pel. noctiluca* has been known for over 100 years (Metchnikoff, 1886; Goette, 1893). In *Pel. noctiluca*, planulae develop into small ephyrae (Fig. 8B). Spawning occurs approximately 2 h after first light exposure (Helm *et al.*, 2015). At 300 μm, *Pel. noctiluca* eggs are among the largest known in Scyphozoa (although still dwarfed by those of *Per. periphylla*; Berrill, 1949), and come in a variety of colours (Helm *et al.*, 2015). The first embryonic cleavage is unipolar and total, and at 18°C embryos become motile at 12 h post-fertilization. Gastrulation is not fully characterized, but involves an invagination at the future oral surface (Helm *et al.*, 2015). As gastrulation progresses, endoderm forms in the oral half of the embryo (Fig. 8Bi). Two to three asymmetrical pockets form within this endoderm, and eventually fuse to form the ephyra gastric cavity (Goette, 1893; Helm *et al.*, 2015). At roughly 60 h post fertilization, four ephyra arm buds are visible on the oral surface around the mouth (Fig. 8Bii). Shortly after this 'four-prong stage', four more arm buds develop interspaced between the first four, to give a total of eight arm buds (Fig. 8Biii). These arm buds develop in a similar manner to those in *Ch. chesapeakei* and *Ch. achlyos* (Helm *et al.*, 2015). Here, ephyra development appears quite

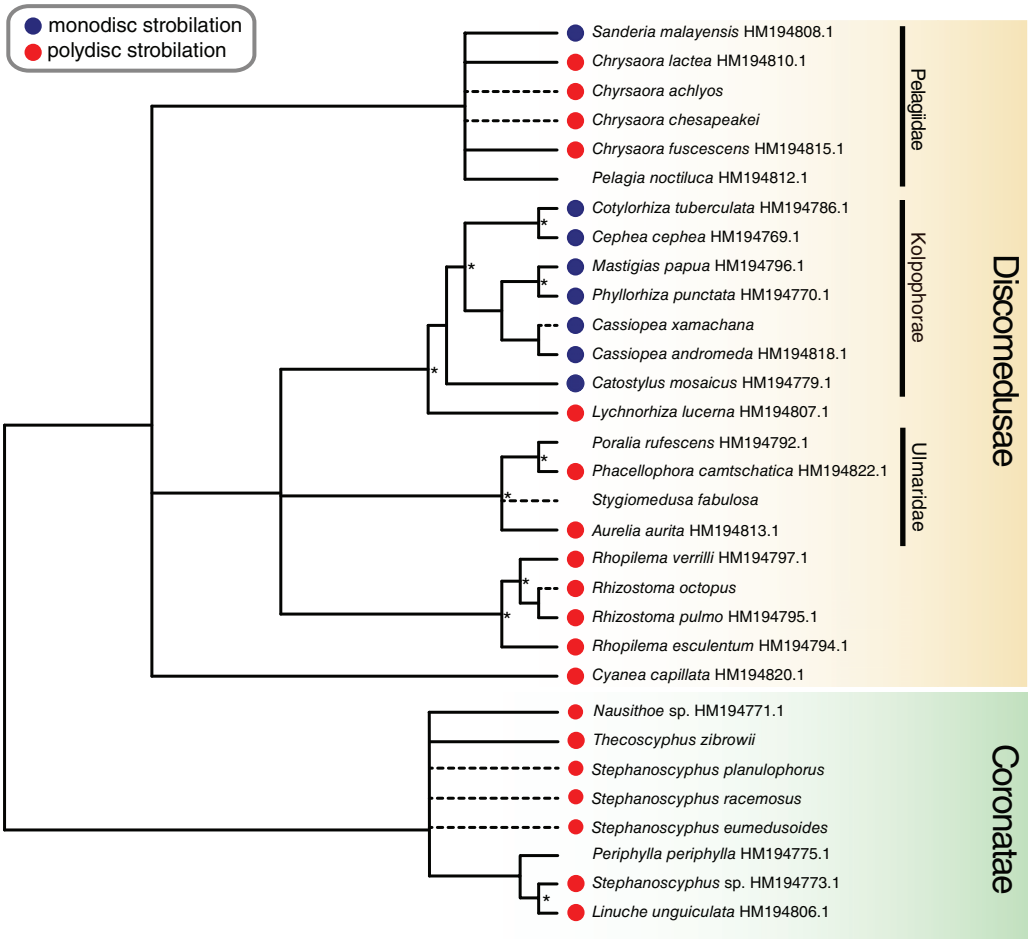

Fig. 6. The distribution of strobilation types in Scyphozoa: polydisc strobilation (red), and monodisc strobilation (blue). Monodisc strobilation appears to have arisen at least twice, once in the last ancestor of Kolpophorae (which includes *Cotylorhiza tuberculata*, *Cephea cephea*, *Cassiopea andromeda*, *Ca. xamachana*, *Catostylus mosaicus*, *Phyllorhiza punctata*, and *Mastigias papua*), and once in the ancestor of *Sanderia malayensis*. Sequences of 18S and 28S ribosomal DNA from Bayha *et al.* (2010) were used to build this tree. Genes were aligned using ClustalW2 with standard input parameters. The topology is based on a consensus maximum likelihood (ML) tree generated with RaxML (v.8) with 100 bootstrap replicates. Bootstrap support values >90 are indicated with an asterisk (*). All species used to generate the base ML tree are indicated with an associated Genbank ID following the species name, and a solid branch. This topology is supplemented with additional species for which there are currently no gene-sequence data. The positions of these species are based on Linnaean taxonomic rank, and indicated with dashed branches. Character states were coded as shown in Table 1.

similar to that of closely related species with strobilation, but begins at the planula rather than the polyp stage (Fig. 8Biv).

Two additional scyphozoan species have been reported to show direct development, although much less is known about them. The deep-water ulmarid *Poralia* sp. may develop directly. Straehler-Pohl, Widmer & Morandini (2011, p. 22) state, 'There are also data suggesting that the ulmarid *Poralia* sp. (Poraliinae) from deep waters of Monterey Bay Canyon is probably a holopelagic, brooding jellyfish, lacking scyphistoma stages in its life cycle (Chad Widmer, unpublished results)'. However, further substantiation has not been published.

A second ulmarid species with a modified life cycle is *Stygiomedusa fabulosa* (Russell & Rees, 1960). In a large specimen collected by Russell & Rees (1960), no mature gonads were found, but a kind of brood chamber was present in the stomach. A strip of tissue within this brood chamber, called the 'germinal line', gives rise to bud-like structures that the authors called 'scyphistomae', although the ontogenetic and evolutionary relationship between these buds and free-living polyps is not clear. Each 'scyphistoma' is contained within a tissue capsule that is connected to the parent medusa. Thus, the developing 'scyphistoma' is secured to the parent stomach in a placenta-like structure. The 'scyphistoma' encased within this odd capsule develops directly into a small medusa, such that 'The most developed capsule, 6.5 cm long and 4.3 cm wide, contained an almost fully developed medusa, complete with gastrovascular system, and mouth arms and the reddish purple coloration typical of the adult' (Russell & Rees, 1960, p. 313). This odd form of development

Table 1. Life-cycle type for a selection of scyphozoans. Life-cycle type refers to either a complex life cycle with planulae, polyps and medusa, or a simplified life cycle missing one or more of these stages. Strobilation type refers to either mono- or polydisc strobilation. Polyp coloniality refers to whether polyps live disconnected from (solitary) or attached to (colonial) other polyps. Different life-cycle stages, including 'Planula', 'Polyp', 'Ephyra', 'Medusa' and 'Medusoid' are coded as present (1) absent (0), or unknown (?). NA, information not applicable.

Clade			Species	Life-cycle type	Strobilation type	Polyp coloniality	Planula	Polyp	Ephyra	Medusa	Medusoid	Source
Coronatae	Pariphyllidae		*Periphylla periphylla*	simple	NA	NA	0	0	0	1	0	Jarms *et al.* (1999)
	Nausithoidae		*Nausithoe aurea*	complex	polydisc	?	1	1	1	1	0	Morandini & da Silveira (2001), Silveira, Morandini & Jarms (2003) and Stampar, Silveira & Morandini (2007)
			Thecoscyphus zibrowii	complex	polydisc	?	1	1	0	0	1	Sötje & Jarms (2009)
			Stephanoscyphus planulophorus	complex	polydisc	solitary	?	1	?	?	?	Werner & Hentschel (1983); Werner (1973)
			Stephanoscyphus mirabilis	complex	polydisc	colonial	?	1	1	1	0	Werner (1970)
			Stephanoscyphus racemosus	complex	polydisc	colonial	?	1	1	?	1	Werner (1970); Werner (1973)
			Stephanoscyphus eumedusoides	complex	polydisc	solitary	?	1	?	0	1	Werner (1973)
	Linuchidae		*Linuche unguiculata*	complex	polydisc	?	1	1	1	1	0	Jarms *et al.* (2002); Puertas *et al.* (2008)
Discomedusae	Cyaneidae		*Cyanea nozakii*	complex	monodisc	solitary	1	1	1	1	0	Dong *et al.* (2008)
			Cyanea capillata	complex	polydisc	solitary	1	1	1	1	0	Gröndahl & Hernroth (1987)
	Pelagiidae		*Chrysaora fuscescens*	complex	polydisc	solitary	1	1	1	1	0	Widmer (2008)
			Chrysaora chesapeakei	complex	polydisc	solitary	1	1	1	1	0	Calder (1974)
			Chrysaora lactea	complex	polydisc	solitary	1	1	1	1	0	Morandini, da Silveira & Jarms (2004)
			Chrysaora melanaster	complex	polydisc	solitary	1	1	1	1	0	R.R. Helm, personal observations
			Pelagia noctiluca	simple	NA	NA	1	0	1	1	0	Metchnikoff (1886) and Goette (1893)
			Sanderia malayensis	complex	monodisc	solitary	1	1	1	1	0	Adler & Jarms (2009) and Uchida and Sugiura (1978)
	Rhizostomeae	Ulmaridae	*Aurelia aurita*	complex	polydisc	solitary	1	1	1	1	0	Russell (1970), Vagelli (2007) and Mayorova, Kosevich & Melekhova (2012)
			Deepstaria enigmatica	?	?	?	?	?	?	1	?	Russell (1967)
			Stygiomedusa fabulosa	simple?	?	?	?	?	?	1	?	
			Phacellophora camtschatica	complex	polydisc	solitary	1	1	1	1	0	Widmer (2006)

Table 1. Continued

Clade	Species	Life-cycle type	Strobilation type	Polyp coloniality	Planula	Polyp	Ephyra	Medusa	Medusoid	Source
	Poralia sp.	simple?	?	?	?	?	?	1	?	Straehler-Pohl, Widmer & Morandini (2011)
Lychnorhizidae	*Lychnorhiza lucerna*	complex	polydisc	solitary	1	1	1	1	0	Schiariti *et al.* (2008)
Catostylidae	*Catostylus mosaicus*	complex	polydisc	solitary	1	1	1	1	0	Pitt (2000)
Rhizostomatidae	*Rhizostoma pulmo*	complex	polydisc	solitary	1	1	1	1	0	Fuentes *et al.* (2011)
	Rhopilema verrilli	complex	polydisc	solitary	1	1	1	1	0	Calder (1973)
	Rhopilema esculentum	compex	polydisc	solitary	1	1	1	1	0	Dong *et al.* (2008)
Stomolophidae	*Stomolophus meleagris*	complex	polydisc	solitary	1	1	1	1	0	Calder (1982)
Kolpophorae	*Cephea cephea*	complex	monodisc	solitary	1	1	1	1	0	Sugiura (1966)
	Colylorhiza tuberculata	complex	monodisc	solitary	1	1	1	1	0	R.R. Helm, personal observations
	Cassiopea andromeda	complex	monodisc	solitary	1	1	1	1	0	Hofmann, Neumann & Henne (1978)
	Mastigias papua	complex	monodisc	solitary	1	1	1	1	0	Sugiura (1964)
	Phyllorhiza punctata	complex	monodisc	solitary	1	1	1	1	0	R.R. Helm, personal observations Rouse & Pitt (2000)

was described from a large, badly damaged animal collected in a net haul from the deep sea, which unfortunately did not preserve well. However, the accompanying photographs, illustrations and descriptions leave no question that medusa development within the gastric cavity is present in *S. fabulosa* (Russell & Rees, 1960). It is not clear if this form of reproduction is sexual or asexual, or if it is the only form of medusa development in this species.

All four direct developers share a similar open-ocean habitat. In Hydrozoa, direct-developing medusae also have an open-ocean distribution (Gibbons *et al.* 2009). The evolution of direct development in Scyphozoa may thus be correlated with this open-ocean distribution, and a lack of suitable substrate on which polyps might settle.

In addition to these cases of obligate direct development, there are several descriptions of facultative direct development in the ulmarid *Aurelia* sp. Haeckel (1881) described some planulae from an *A. aurita* culture that developed directly into tiny ephyrae. Kakinuma (1975) also documented ephyrae development from planulae in *A. aurita*, and reported that overcrowding of planulae, plus the presence of parental mucus, caused some planulae to develop directly into ephyrae. If *A. aurita* can indeed develop ephyrae either directly or indirectly, this would provide an excellent comparative system to contrast with both obligate direct and indirect developers.

In contrast to the loss or modification of planulae and polyps, a small subset of scyphozoans have reduced or lost the medusa stage. 'Medusoids' – highly reduced medusae – are reported in *Stephanoscyphus racemosus* and *Stephanoscyphus eumedusoides* (Werner, 1974; Jarms *et al.*, 2002). *Thecoscyphus zibrowii* shows the most reduced medusa development of any scyphozoan known to date (Sötje & Jarms, 2009). Polyps form oocytes that are then encased in an egg sac during a process that resembles strobilation, with the egg sac being a greatly reduced medusa. The egg sac is not liberated, and planulae eventually burst from it, after which the reformed polyp pushes the egg sac out of its chitinous tube (Sötje & Jarms, 2009).

In the cave-dwelling *Stephanoscyphus planulophorus*, ephyrae rudaments did not develop into sexually reproductive medusae but instead metamorphosed into ciliated planuloids (Werner & Hentschel, 1983). However, it is not clear whether this stage is facultative; as mentioned previously, another coronate, *Linuche unguiculata*, can produce both planulae and medusae *via* strobilation (Silveira & Morandini, 1998) (Fig. 3C). Additionally, when *Aurelia* sp. (and *Ch. chesapeakei*, personal observations) strobilae are exposed to major environmental stress (e.g. extremes in temperature or salinity) they can become 'polyp-strobilae,' whereby each segment develops not into an ephyra, but into a polyp (as reviewed in Spangenberg, 1968) (Fig. 3D, E). It is possible that *Stephanoscyphus planulophorus* produce planuloids or medusoids under certain conditions, and ephyrae under others. It is also possible that non-ephyra strobilation may have become developmentally canalized in this species, and is the only form of strobilation present.

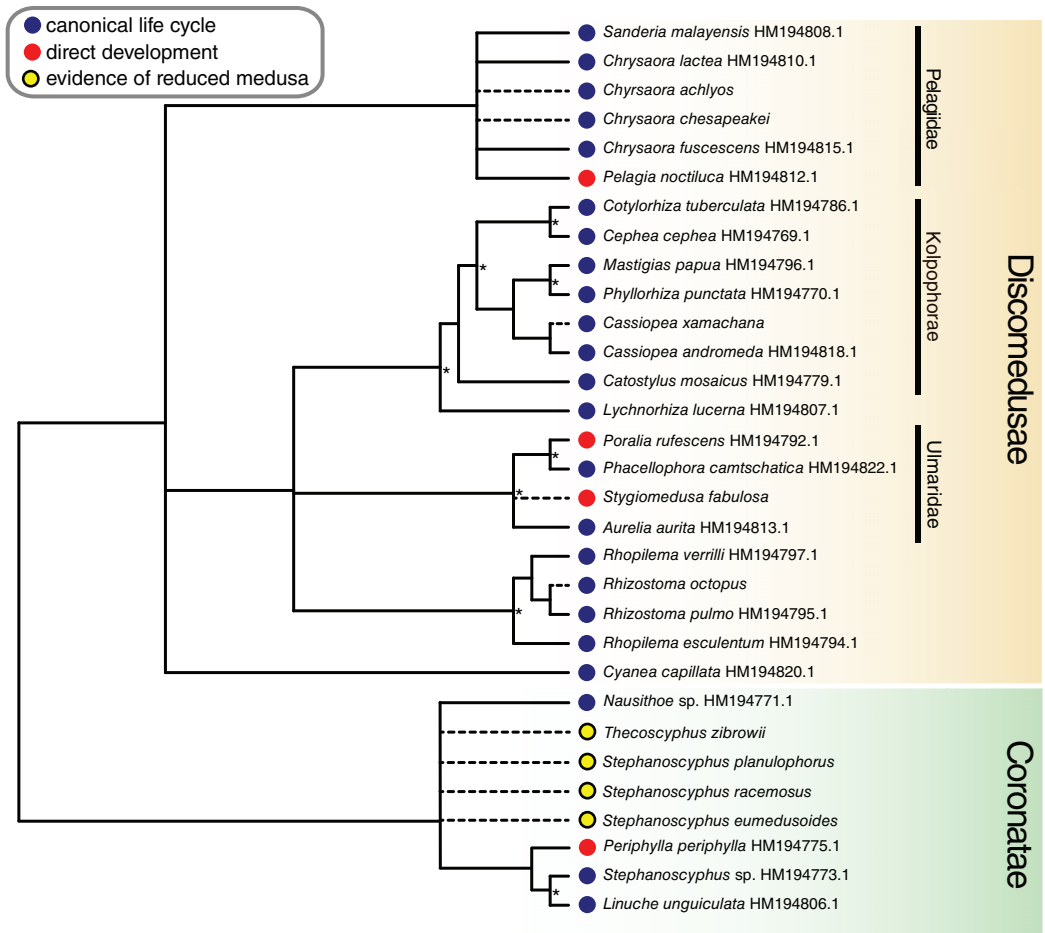

Fig. 7. The distribution of life-cycle types in Scyphozoa. Direct ephyra/medusa development appears to have originated at least three times independently (probably four, but no sequence data are available for *Stygiomedusa fabulosa*). Reduced medusa development is present in the Coronatae. Sequences of 18S and 28S ribosomal DNA from Bayha *et al.* (2010) were used to build this tree. Genes were aligned using ClustalW2 with standard input parameters. The topology is based on a consensus maximum likelihood (ML) tree generated with RaxML (v.8) with 100 bootstrap replicates. Bootstrap support values >90 are indicated with an asterisk (*). All species used to generate the base ML tree are indicated with an associated Genbank ID following the species name, and a solid branch. This topology is supplemented with additional species for which there are currently no gene-sequence data. The positions of these species are based on Linnaean taxonomic rank, and indicated with dashed branches. Character states were coded as indicated in Table 1.

VI. ECOLOGICAL CUES AND STROBILATION

External environmental conditions often play a key role in triggering strobilation. I now briefly review the environmental variables involved in the induction of strobilation. A more detailed treatment can be found in Lucas *et al.* (2012). Although several environmental parameters, such as temperature, are important in many species, the exact cues to strobilation induction appear variable both within and among species, and likely reflect local and historical selective pressures.

Strobilation in many species is triggered by abiotic factors associated with seasonal changes. In the temperate species studied to date, temperature is important in strobilation induction, although the temperature and duration of change necessary may vary with species and population (Lucas *et al.*, 2012). For example, many populations of *A. aurita* produce ephyrae after prolonged cold periods, such as in Kertinge Nor Fjord, Denmark, where ephyrae first appear in February (Olesen & Riisgaard, 1994). However, in Gullmar Fjord in western Sweden, *A. aurita* polyps strobilate in October (Gröndahl, 1988). These two different populations may be responding to different environmental cues, including temperature, or may require different durations of cold before strobilation. This may also be due to cryptic species variation in *A. aurita* (Dawson & Jacobs, 2001), genetic variation between populations, interannual variation, variation in environmental conditions between the two sites, or some combination thereof. *Chrysaora chesapeakei* in Chesapeake Bay produce ephyrae in the late spring and summer (Cargo & Rabenold, 1980), while *Cy. capillata* in the same area are reported to produce ephryae in the autumn

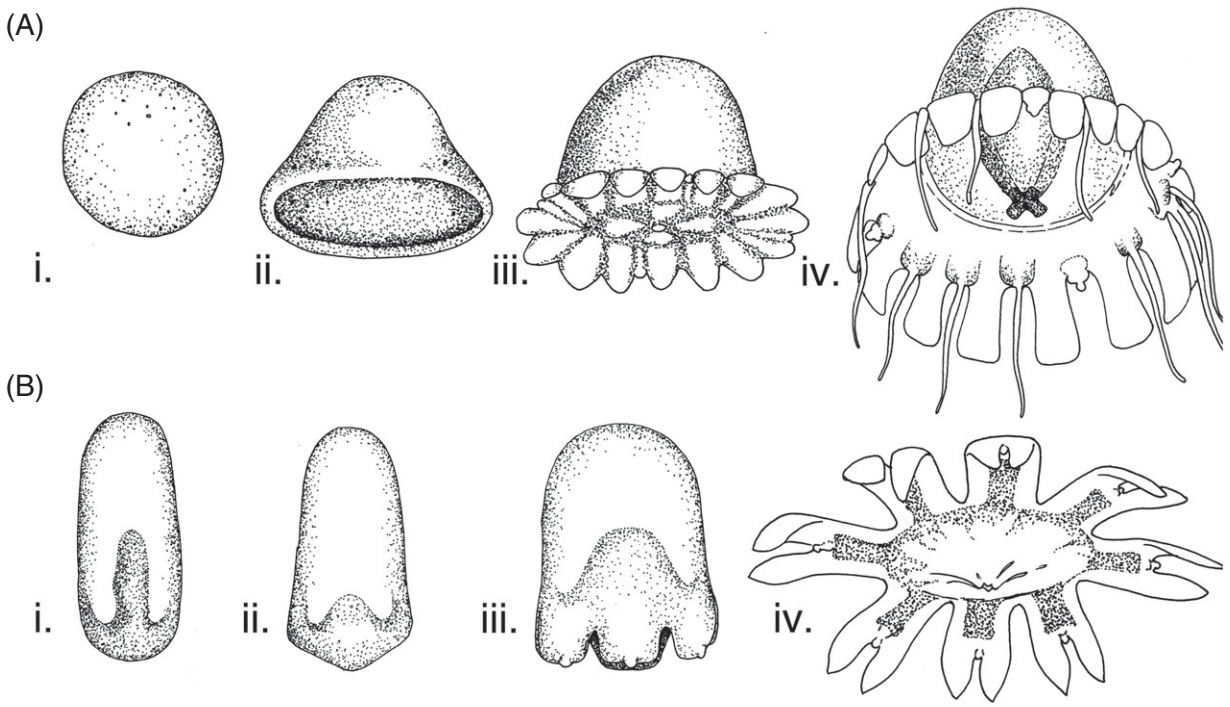

Fig. 8. Development of two scyphozoan species without a polyp stage. (A) Development of *Periphylla periphylla* larvae, based on Jarms *et al.* (1999): (i) a large yolky egg is fertilized; and (ii) develops into a thimble shaped larva; (iii) over time, this thimble-larva grows rhopalar arms on the oral surface; and (iv) eventually becomes a young medusa, bypassing the planula and ephyra stages. (B) Development of *Pelagia noctiluca* begins when (i) a planula with a core endoderm develops into (ii) a 'four-prong larvae' with four developing rhopalar arms on the oral epidermis. (iii) Slightly later, this larva develops four additional arm buds with small rhopalia on the tips. (iv) These arm buds develop into swimming arms in mature ephyrae, each with two rhopalial lappets, one on each side of a rhopalium.

(Cargo & Schultz, 1967). For *R. octopus*, strobilation is induced by both an increase or decrease in temperature (Holst *et al.,* 2007). Some Kolpophorae species strobilate in response to increased temperatures (Holst *et al.,* 2007).

Salinity and light exposure may also play important roles in strobilation (Lucas *et al.,* 2012). Changes in salinity affect the onset of strobilation in *Ch. chesapeakei* (Purcell *et al.,* 1999). *Chrysaora chesapeakei* is an estuarine species, and fresh water may be an important seasonal trigger. Light intensity and photoperiodicity may also play crucial roles in inducing strobilation in some species (Purcell *et al.,* 2007; Purcell, Hoover & Schwarck, 2009). However, a possible role of light in inducing strobilation remains unclear (Lucas *et al.,* 2012). In some species with symbiotic zooxanthellae in the polyps and medusae, the zooxanthellae appear to be required for strobilation, as polyps uninfected with zooxanthellae do not strobilate (Hofmann & Kremer, 1981).

VII. MOLECULAR MECHANISMS OF STROBILATION

The biochemical processes involved in strobilation are not well understood. Limited observations have been made on structural neuronal changes associated with strobilation (Arai, 1997), and several compounds are known either to induce or to be associated with strobilation.

Little is known about neuronal features associated with strobilation. Loeb & Hayes (1981) investigated features of polyp neurons in and around the calyx of *Ch. chesapeakei*. In pre-strobila polyps and early strobilae, but not in young polyps, these calyx neurons show evidence of neurosecretory material, which was secreted into the surrounding tissue in early strobilae. Neurosecretory cells containing neurosecretory granules have also been observed in *Ch. chesapeakei* polyps at the neck-formation stage (Crawford & Webb, 1972). In segmenting strobilae, dense granules are found in axons, but these are largely absent in strobilae at later stages. These secreted compounds may play a role in strobilation induction or progression.

Environmental compounds are also known to have an important role in strobilation. Iodine, a trace element in sea water, is required for strobilation (Paspalev, 1938): when kept in iodine-free sea water, polyps of *Rhizostoma pulmo* do not strobilate. Spangenberg (1967) studied a Texas strain of *A. aurita* in which cold periods are necessary for strobilation, and found that even prolonged cold in the absence of iodine was insufficient to induce strobilation. However, adding

iodine to polyps 'conditioned' in cold water caused a rapid onset of strobilation. This suggests that iodine may function in a temperature-induction pathway (Spangenberg, 1967). Using radioautography, Spangenberg (1971) showed that radioactive iodine was incorporated into patches of cells in the polyp ectoderm and gastroderm, as well as the mesoglea. In another study on *A. aurita*, iodine concentrations were reportedly three times higher in the segmented region of a strobila compared to the aboral foot (Olmon & Webb, 1974) and the authors suggested that inorganic iodide, rather than bound iodine, was responsible for induction. Iodine is also necessary for strobilation in *Ch. chesapeakei* (Black & Webb, 1973).

Spangenberg (1967) also found that the addition of thyroxine, a vertebrate hormone involved in thyroid signalling, induced strobilation in cold-treated polyps. Iodine-containing intermediates in the thyroid hormone synthesis pathway, including triiodothyronine, diiodotyrosine (DIT), monoiodotyrosine (MIT), and thyroglobulin, had a similar effect (Fig. 9). All of these compounds contain iodine, and DIT induced strobilation in as little as 4 days, i.e. a rate comparable to elemental iodine (Spangenberg, 1971). Using Radiochromatography, Spangenberg (1971) identified three endogenous protein-bound iodinated compounds in *A. aurita* polyps after iodine induction. One of these compounds is secreted, and Spangenberg (1971) tentatively identified it as thyroxine. The other two compounds were tentatively identified as MIT and DIT (Spangenberg, 1974), explaining why ectopically applied thyroxine, DIT, and MIT induced strobilation. Putative MIT was found 8 h after iodide addition, while putative thyroxine was found between 16 and 24 h and putative DIT after 24 h (Spangenberg, 1974). Similarly to *A. aurita*, two compounds tentatively identified as iodotyrosines and thyroxine were identified in *Ch. chesapeakei* (Black & Webb, 1973). Spangenberg (1974) showed that the addition of iodine concurrently with goitrogens (compounds that interfere with thyroid function in mammals by inhibiting iodine uptake) to *A. aurita* polyp cultures failed to induce strobilation. Chromatography confirmed that the addition of these goitrogens inhibited iodine uptake, as well as the synthesis of the three protein-bound iodine compounds.

The fact that iodine and thyroid hormone-like compounds may be involved in strobilation in the two species, *A. aurita* and *C. chesapeakei*, is phylogenetically significant as these species are in two different clades (Fig. 1): *Chrysaora* is in Pelagiidae, while *Aurelia* is in Rhizostomeae + Ulmaridae. Shared features between these two groups thus suggest that these features may have been present in the most recent common ancestor of Discomedusae. While additional data are needed (particularly from Cyaneidae and Coronatae), iodine and thyroid hormone-like compounds may be necessary in strobilation for all Discomedusae, and potentially all scyphozoans.

Rather than an active role for thyroid hormone in strobilation induction, Berking *et al.* (2005) suggested that iodine functions in a larger oxidative defence pathway, and it is this defence pathway that induces strobilation. The authors suggest that iodide in sea water and reactive oxygen species (ROS) interact to form iodine, and this iodinates an unknown target, which induces strobilation. The authors added hydrogen peroxide to sea water, and induced strobilation in *Aurelia* sp., suggesting that ROS may indeed be involved in strobilation induction.

Berking *et al.* (2005) also found that the addition of tyrosine inhibited strobilation, and suggested that tyrosine is a natural strobilation inhibitor. Tyrosine is iodinated by iodine, and the authors suggest that this iodinated tyrosine acts as a natural sink for iodine: ROS and iodide will continuously produce low levels of iodine, which binds to tyrosine and diffuses into the sea water, removing iodine from the system. However, when tyrosine levels are reduced in polyp tissue, or the iodine/ROS ratio is increased, iodine can then react with an unknown strobilation-induction molecule. Once strobilation is initiated, the authors suggest that additional segments are formed in polydisc strobilators through a reduction of tyrosine levels in nearby tissue. Many *Aurelia* sp. lines produce pigmented ephyrae, and the authors propose that the production of melanin in the topmost segments, which requires tyrosine, may reduce tyrosine levels in lower segments; this reduction in tyrosine then causes the polyp tissue to form ephyra tissue.

Berking *et al.* (2005) added compounds that inhibit melanin production in other organisms (D-penicillamine and caffeic acid) to strobilae, and halted the process of strobilation. Following addition of D-penicillamine, strobilae transform to stacks of polyps. However, as mentioned in Section V, strobilae exposed to stress can form polyp-strobilae. Furthermore, the presence of melanin as a pigment in strobilae has not yet been verified. While the Berking *et al.* (2005) model is compelling, it requires further testing, in particular, regarding the identification of the proposed iodinated target, and replication of their observations on hydrogen peroxide induction.

Two studies also report that unwashed polystyrene dishes are effective inducers of strobilation. In the Discomedusa *A. aurita*, 69 animals strobilated in unwashed dishes out of 144, compared to 0 out of 144 in washed dishes (Herrmann, Siefker & Berking, 2003). In the Coronatae *Nausithoe aurea*, six of eighteen polyps strobilated in unwashed dishes, compared to zero of eighteen in washed dishes (Stampar, Silveira & Morandini, 2007). The particular compounds triggering metamorphosis are unknown, though may be related to oxidative stress (Herrmann, Siefker & Berking, 2003). Given the simplicity of the protocol, and potential efficacy in both discomedusans and cornonates, additional studies are warranted.

Several authors have attempted to characterize proteins secreted in association with strobilation. Concentrating sea water in which polyps were induced to strobilate allowed Loeb (1974*a*) to identify a 'neck-inducing factor (NIF)' that is secreted several hours after *Ch. chesapeakei* are exposured to induction cues. Loeb (1974*b*) identified this NIF as a 1650 Da peptide, and polyps exposed to monomers, dimers

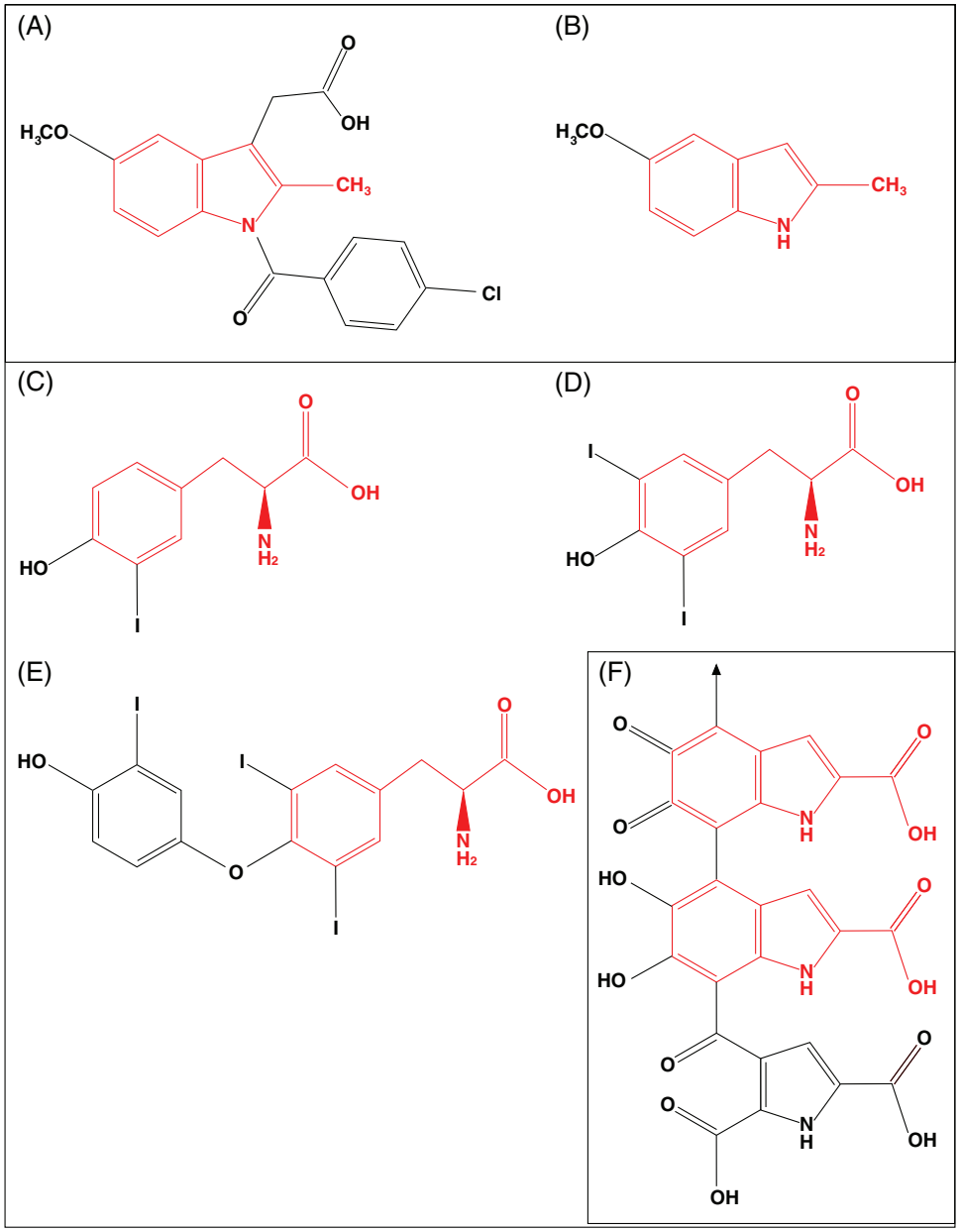

Fig. 9. Some compounds reported to induce strobilation. Red represents regions of potential functional significance, as determined by the efficacy of synthetic indole-containing compounds (Fuchs *et al.*, 2014). (A) Indomethacin and (B) 5-methoxy-2-methylindole induce strobilation in a broad diversity of Discomedusae (Kuniyoshi *et al.*, 2012; Fuchs *et al.*, 2014; Helm & Dunn, in press). (C–E) Thyroid hormone and precursors are effective in inducing strobilation in an *Aurelia aurita* line from Texas (Spangenberg, 1971, 1974): (C) monoiodotyrosine (MIT), (D) diiodotyrosine (DIT), and (E) triiodothyronine (T3). (F) Eumelanin has been implicated in strobilation in *A. aurita* (Berking *et al.*, 2005).

and tetramers of this peptide formed elongated necks. The identity of the NIF is unknown.

For *A. aurita* polyps from Roscoff, France, the onset of strobilation after exposure to diffusible endogenous molecules is quite rapid. Fuchs *et al.* (2014) showed that strobilation can be induced by exposing *A. aurita* polyps to strobila tissue through either ingestion or physical contact. Feeding pieces of strobilae to polyps induced strobilation in as little as 48 h. However, this was only the case for contact or ingestion of strobila pieces from the same culture line. Exposing polyps to strobila tissue from other polyp lines or populations did not induce strobilation (Fuchs *et al.*, 2014).

While thyroid hormone-like compounds have been implicated as diffusible inducers of strobilation (Spangenberg, 1967), Fuchs *et al.* (2014) suggest a different signalling pathway. Fuchs *et al.* (2014) examined gene expression during strobilation in a Roscoff strain of *A. aurita*. They discovered that several possible homologues of genes associated with

retinoic acid (RA) signalling were differentially expressed during strobilation, including two retinol dehydrogenases (RDHs) and a homologue of retinoid X receptor (RxR). One RDH was downregulated during strobilation, while the other RDH and RxR were both upregulated. RDHs are enzymes that convert vitamin A (retinol) into the intermediate 9-cis RA, which then binds to a heterodimer complex including RxR. Incubating polyps in 9-cis RA induced strobilation after 7–12 days, considerably faster than the 19–21 days necessary for temperature induction. By contrast, exposure of polyps to two chemicals that inhibit retinoic acid signalling, 4-diethylaminobenzaldehyde (DEAB) and UVI3003, delayed strobilation with 9-cis RA. However, DEAB did not inhibit naturally induced strobilation. By contrast, UVI3003 delayed (1 μM) or fully repressed (5 μM) naturally induced strobilation. Together, these data suggest that RA signalling may be involved in strobilation induction. However, inducing polyps with 9-cis RA was still considerably slower than feeding polyps pieces of strobilae (Fuchs *et al.*, 2014) implying that 9-cis RA does not act alone.

Fuchs *et al.* (2014) also identified transcripts that are strongly upregulated in strobilae. One such transcript, CL390 (short for 'cluster 390' on their microarray), was only expressed in strobilae. This transcript increased with cold-temperature exposure, and Fuchs *et al.* (2014) suggested that it may act as a cold-sensitive molecular timer by increasing during cold exposure until a threshold is reached, after which strobilation is induced. To test the function of CL390, Fuchs *et al.* (2014) synthesized short peptides based on the transcript sequence. One peptide sequence (WSRRRWL) induced strobilation when polyps were soaked in a peptide seawater solution. However, this response may be species-specific.

Brekhman *et al.* (2015) sequenced mRNA from an *A. aurita* line from the Red Sea. A CL390-like transcript was identified that appeared to be upregulated during strobilation. Interestingly, they found that this CL390-like transcript showed only 69% similarity to the Roscoff *A. aurita* CL390 sequence, and was missing the peptide sequence characterized by Fuchs *et al.* (2014). Such a high degree of variation suggests rapid evolution of this protein, or may be related to the considerable population-level variation in strobilation onset. This could explain why polyps only strobilate when fed strobila pieces from the same line (Fuchs *et al.*, 2014). Additional CL390 sequences from other *A. aurita* lines and scyphozoans are needed to examine these hypotheses further.

Several other strobilation-inducing small molecules have also been identified. The drug indomethacin was found to induce strobilation in an *A. aurita* population from southern Japan (Kuniyoshi *et al.*, 2012). The core structure of indomethacin is an indole, an aromatic bicyclic structure composed of two adjoined rings: a six-membered benzene and a nitrogen-containing five-atom ring (Fig. 9). Kuniyoshi *et al.* (2012) found that indomethacin induces strobilation in a dose- and duration-dependent manner. Fuchs *et al.* (2014) identified four additional indole-containing compounds that induced strobilation in the Roscoff *A. aurita* line. They tested a diversity of indoles, but only those modified with a methyl or carboxylic group at the C2 position induced strobilation.

Keeping indoles in mind, recall that Berking *et al.* (2005) found that pharmacological inhibition of melanin production halts strobilation. A common form of melanin, eumelanin, is composed of multiple linked indoles, all with a carboxylic group at the C2 position (Fig. 9). Pharmaceuticals that inhibit melanin production thus could potentially react with other indole pathways. Triiodothyronine, DIT, and MIT also all show structural similarity, with carboxylic groups at C2 (Fig. 9), and CL390 has several tryptophans with associated indole rings. Perhaps the ability of triiodothyronine, DIT, MIT, and CL390 to induce strobilation is related to their structural similarity to endogenous indoles, or *vice versa*.

Several authors have used indoles to induce strobilation in a variety of scyphozoan species (Yamamori *et al.*, 2017; Helm & Dunn, 2017). Nearly all discomedusan scyphozoans tested strobilate in the presence of indoles, but the coronate *Linuche* sp. does not (Helm & Dunn, 2017). Because the same compounds induce the same response across multiple species sharing a common ancestor, it is likely that indoles may be targeting an evolutionarily conserved endogenous signalling pathway, at least in Discomedusae. Indoles are abundant in the marine environment, and in addition to the possibility that polyps may produce indoles or structural analogues endogenously, it is also possible that polyps sense these compounds (such as from prey items, algae, or bacteria) and use these cues to strobilate. Regardless of where indoles or structural analogues originate, the responsiveness of polyps to indoles appears highly conserved (Helm & Dunn, 2017).

VIII. FUTURE DIRECTIONS

Many unanswered questions remain on the development and evolution of scyphomedusae. For many species, we do not even have basic information on their life histories. Obtaining this information will not only enhance our understanding of life-cycle evolution and development, but may also provide valuable ecological insights.

For species where the full life cycle is known, we have an opportunity to explore more in-depth questions on evolution and development. For example, to what extent are polyp morphologies remodelled during ephyrae formation? Generally, there are two major categories of metamorphosis in animals: remodelling, where larval tissues are reformed into adult tissues, and compartmentalization, where larval tissues degrade and adult tissues develop from quiescent cells set aside during embryogenesis for the development of adult tissues (Moran, 1994). In many organisms, such as holometabolous insects or sea urchins, many adult tissues are derived from set-aside cells. However, in these cases, metamorphosis is rapid and identifiable quiescent cell populations are present in the larvae (e.g. imaginal discs and rudiment cells). No clear multipotent or

quiescent cells are evident in Scyphozoa (Gold & Jacobs, 2012). Another possible mechanism of metamorphosis is cellular reprogramming, wherein previously differentiated cells de- or trans-differentiate to form new tissues. Evidence for this phenomenon has been observed in scyphozoan embryonic development (Yuan *et al.*, 2008; Gold *et al.*, 2016).

There is some evidence that metamorphosis *via* strobilation involves the formation of new tissues in ephyrae, rather than remodelling of polyp tissue. Ephyra muscle is not remodelled from pre-existing polyp muscle in *Ch. chesapeakei* (Helm *et al.*, 2015). The same is true for tentacles; rather than medusa tentacles being remodelled from pre-existing polyp tentacles, polyp tentacles are resorbed and new medusa tentacles develop separately (Spangenberg, 1968; Gold *et al.*, 2015). Polyp-type kinocilia are also replaced by ephyra-type kinocilia at the site of future rhopalia (Spangenberg, 1991), and polyp atrichous polyspiras nematocysts are degraded during strobilation (Spangenberg, 1968). However, as an exception, Schwab (1977*b*) suggests that the polyp nervous system may be partially retained in ephyrae. Combined, these data suggest that many polyp features are lost during metamorphosis, rather than remodelled or co-opted, but that some morphological features may persist across life-history stages.

However, many unknown questions remain: if it is indeed found that most tissues in developing ephyrae are not remodelled from similar polyp tissue, what is the origin of the cells giving rise to these new medusa-specific tissues? Do polyps have a cryptic population of cells reserved for medusa development, or do polyp cells de-differentiate or trans-differentiate to form medusa tissues? What cell populations become the medusa gonadal tissue and gametes? Is the germ line set aside in polyps, or does it form from previously differentiated tissue?

It is thus essential to investigate whether medusae are remodelled polyps or whether polyps and medusae are developmentally distinct and arise from different embryonic tissue sources. This will provide valuable information for the comparative study of metamorphosis across Scyphozoa, and more broadly across animals.

In addition to questions related to life-cycle evolution, there are also many basic questions about ephyrae development that have not been thoroughly explored. For example, the morphogenesis of medusa structures like gastric pockets and their enigmatic gastric filaments is largely undescribed.

Perhaps some of the most pressing questions in the evolution and development of scyphomedusae are related to strobilation induction. While thyroid hormone-like compounds and 9-cis RA have been implicated in strobilation induction (Spangenberg, 1971, 1974; Fuchs *et al.*, 2014), none of these compounds have been conclusively identified in scyphozoans. Both retinoic acid and thyroid hormone are ligands for an important class of proteins called nuclear hormone receptors. Small signalling molecules and nuclear hormone receptors initiate metamorphosis in animals ranging from insects to frogs. If nuclear hormone receptors, including RxR, are involved in strobilation, this suggests that the role of these proteins in metamorphosis dates back to the last common ancestor of Cnidaria and Bilateria (Fuchs *et al.*, 2014).

On an ecosystem level, understanding the molecular cues involved in strobilation may help to predict jellyfish blooms. If molecular cues, such as indoles or secreted compounds (such as NIF or CL390-like peptides), are sensed by polyps, these compounds could be used to monitor bloom potential.

Most studies on strobilation induction have been conducted on different lines of one presumptive species (*A. aurita*) or in *Ch. chesapeakei*. Expanding our knowledge to a wider range of species, particularly by including coronates, will provide a broader perspective on strobilation induction and scyphomedusa development. There is increasing evidence that *A. aurita* is actually a species complex with a global distribution (Dawson & Jacobs, 2001), and different polyp populations are known to strobilate in response to different environmental conditions (Olesen & Riisgaard, 1994). The gene and protein variants that might be related to this population-level variation in environmental responsiveness remain unknown. Feeding experiments, where polyps are induced to strobilate by ingesting pieces of ephyra tissue, may provide some insights. As discussed above (Section VII), Fuchs *et al.* (2014) observed that only polyps fed ephyra fragments from the same population strobilated, suggesting species and/or population-level genetic differences in strobilation induction. Gene expression and sequence variation of candidate genes like CL390 may provide early clues about niche adaptation and strobilation.

Understanding how different marine populations vary in environmental responsiveness goes beyond simply understanding scyphozoan biology. Earth's climate is changing, and populations must respond to these changes. With increasing ship-assisted transport of species around the world, different *Aurelia* spp. have been (Greenberg, Garthwaite & Potts, 1996), and will likely continue to be, introduced to new locations. There is little understanding of how native and introduced scyphozoan populations respond to changing environments.

Identifying conserved molecular components of the strobilation induction pathway is a critical first step in understanding life-cycle evolution in Scyphozoa. Are genes associated with strobilation onset also present in direct developers, and if so, when are they expressed? How does the initiation of ephyra development in obligate direct developers compare with that of facultative direct developers? Answering these questions will provide greater insights not only into the evolution of scyphomedusae, but also into the evolution and development of complex life cycles in general.

The relationship between segmentation and ephyra development is also not well understood. Segmentation can give rise to ephyrae, but also to polyps and planuloids. It is not clear whether the molecular cues associated with strobilation

relate specifically to ephyra strobilation or whether they are associated more generally with segmentation.

This issue also relates to the fact that scyphozoans exhibit both convergent development – where distinct developmental starting points give rise to the same developmental outcome (such as the uppermost ephyra disc in polydisc strobilators, and in facultative direct and indirect ephyrae development) – and divergent development – where similar developmental starting points can give rise to different developmental outcomes (such as strobila discs forming either ephyrae or polyps within the same species). These unique features of scyphozoan development provide a unparalleled opportunity to study the interplay of canalization and plasticity in development.

There is still much work to be done on the development and evolution of scyphomedusae. Continuing to investigate the developmental processes that give rise to medusae will provide key insights into the biology, ecology, and evolution of these fascinating organisms.

IX. CONCLUSIONS

(1) Strobilation is the primary means of ephyra generation. Early signs of ephyra formation include thickening at the base of polyp tentacles in some species, the formation of an elongated body column or 'neck', and development of the first segmentation furrow. In species with polydisc strobilation, the topmost ephyra and subsequent ephyrae develop from different types of polyp tissue.

(2) Polydisc strobilation is likely ancestral, with monodisc strobilation having arisen once in Kolpophorae, and once in *Sanderia malayensis*.

(3) There are at least three, possibly four, examples of independent loss or modification of planula/polyp stages. These all occur in medusae with an open-ocean distribution, suggesting that the loss of a benthic polyp is correlated with an offshore lifestyle. There is also one case of facultative direct development.

(4) In several cases, medusae are highly reduced or possibly lost. All of these occur within the Coronatae lineage, but it is currently unclear if these species form a monophyletic group. In several scyphozoans, strobilae produce polyps (observed in Discomedusae) or planuloids (observed in Coronatae), possibly in response to stress. The loss of a medusa stage in some coronates may be due to particular environmental cues, or to evolutionary canalization of environmentally specific responses.

(5) Multiple ecological cues can trigger strobilation and ephyrae production in a population- or species-specific manner, particularly abiotic factors associated with seasonal changes.

(6) Molecular and cellular processes associated with strobilation and ephyrae production include changes in polyp neuronal anatomy at the onset of strobilation, secretion of unidentified diffusible proteins, and a physiological requirement for elemental iodine. Hydrogen peroxide, thyroid hormone pathway members (including thyroxine, triiodothyronine, diiodotyrosine, monoiodotyrosine and thyroglobulin), indomethacin and other similar indoles, 9-cis retinoic acid, neck-inducing factor, and small fragments of the protein CL390 have all been reported to induce strobilation in some species. The requirement for iodine appears to be evolutionarily conserved, and several different hormones (including thyroid hormone and retinoic acid) have been implicated in strobilation induction. Indoles also induce strobilation in a broad range of Discomedusae, suggesting that the cellular processes involved in strobilation induction are evolutionarily conserved across a diversity of species.

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